Targeting the Cancer Initiating Cell: The Ultimate Target for Cancer Therapy

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Abstract: An area of therapeutic interest in cancer biology and treatment is targeting the cancer stem cell, more appropriately referred to as the cancer initiating cell (CIC). CICs comprise a subset of hierarchically organized, rare cancer cells with the ability to initiate cancer in xenografts in genetically modified murine models. CICs are thought to be responsible for tumor onset, self-renewal/maintenance, mutation accumulation and metastasis. CICs may lay dormant after various cancer therapies which eliminate the more rapidly proliferating bulk cancer (BC) mass. However, CICs may remerge after therapy is discontinued as they may represent cells which were either intrinsically resistant to the original therapeutic approach or they have acquired mutations which confer resistance to the primary therapy. In experimental mouse tumor transplant models, CICs have the ability to transfer the tumor to immunocompromised mice very efficiently while the BCs are not able to do so as effectively. Often CICs display increased expression of proteins involved in drug resistance and hence they are intrinsically resistant to many chemotherapeutic approaches. Furthermore, the CICs may be in a suspended state of proliferation and not sensitive to common chemotherapeutic and radiological approaches often employed to eliminate the rapidly proliferating BCs. Promising therapeutic approaches include the targeting of certain signal transduction pathways (*e.g*., RAC, WNT, PI3K, PML) with small molecule inhibitors or targeting specific cell-surface molecules (*e.g*., CD44), with effective cytotoxic antibodies. The existence of CICs could explain the high frequency of relapse and resistance to many currently used cancer therapies. New approaches should be developed to effectively target the CIC which could vastly improve cancer therapies and outcomes. This review will discuss recent concepts of targeting CICs in certain leukemia models.

Keywords: Therapeutic sensitivity, targeted therapy, PI3K, PTEN, Akt, mTOR.

OVERVIEWS OF CANCER, STEM CELLS AND CANCER INITIATING CELLS (CICS)

Cancer is a complex, multigenetic disorder characterized by uncontrolled proliferation, blockage in cellular differentiation, and metastases. Previously cancer biologists explained the process of cancer initiation and progression by means of a clonal/stochastic model. According to this model, a cell or a group of cells become tumorigenic after an initial somatic or germinal mutation, and subsequent series of genetic/epigenetic mutations, leading to proliferative and survival advantage of certain cell clones. This theory hypothesizes that the predominant clones with the capacity to produce identical neoplastic cells engender the tumor with the ability to produce initial neoplastic cells which maintain the tumor and capability to initiate other tumors after transplantation [1]. This clonal evolution model assumed that cancer was comprised of a clone or group of clones with similar growth rates that displayed a homogenous morphological pattern. Moreover this theory predicted that all cancer cells could randomly initiate and propagate the tumor by themselves [2-3]. However, this model has two fundamental limitations. First, not all neoplastic cells in a given tumor are homogenous and the differentiation patterns in tumors can be distinguished. Second, the clonal/stochastic model implies that every cancer cell could initiate a tumor. However, this tenant was not consistent with the observation that a large number of cells were required to transfer a tumor.

Before we discuss cancer stem cells, we need to first very briefly describe normal stem cells. Stem cells are often defined by their potential for self-renewal and by their ability to proliferate and differentiate into diverse cell types. Since this review will cover in detail leukemia CICs, we will discuss hematopoietic stem cells (HSC). Normal HSCs have been characterized over the past 50 years [4]. HSCs comprise a very small, but essential, subpopulation of the total hematopoietic cell component, making up less than 0.01% of cells in the bone marrow of a normal individual [5]. Stem cells are also present in other tissue types (*e.g*., breast, prostate and other cell types), but often not as well characterized as HSCs.

A critical breakthrough in cancer research over the past 15 years has been the discovery of cancer initiating cells (CICs). These cells are also sometimes referred to as cancer stem cells; however, the adjective stem is now more frequently replaced with initiating as they lack certain key properties which true stem cells possess. Furthermore, the CICs may have suffered critical mutations which hopefully the "normal" stem cell lacks. The CIC concept entails that cancer initiation and propagation are driven by small subpopulations of cells displaying stem cell-like properties, such as the capability of self-renewal, asymmetric cell division and differentiation. CICs may arise from a normal stem cell and undergo epigenetic changes. CICs are likely capable of differentiating into a phenotypically diverse progeny which may ultimately form subsets of non-tumorigenic bulk cancer (BC) which have a limited capacity to

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divide and survive. CICs are resistant to conventional cytotoxic treatments. This resistance contributes tumor metastases and relapse. Conventional anti-tumor treatments, such as polychemotherapy, can only act on more mature BCs whereas CICs are normally resistant to these approaches, often due to the overexpression of proteins involved in drug resistance (see below) and their low proliferation rate.

CICs have been identified over the last decade in diverse cancer types and some of the key genes and signaling pathways responsible for their important cancer initiating and invasive properties are beginning to be elucidated [6-12].

Over the last 15 years, the CIC theory of cancer evolution has been proposed. This alternative model assumes the existence of a hierarchical order where a tissue-specific, programmed or reprogrammed cell, the so-called CIC, acquires or retains the properties of self-renewal, multi-lineage differentiation, and most importantly, tumor initiation, both *in vitro* and *in vivo* [13]. This population of rare cells is the only one capable of initiating and maintaining the tumor, thus allowing cancer diffusion to other organs, or cancer transplantation into other living beings, typically mice. According to the CIC model, the BCs would be transient amplifying cells and mature cells with limited or no ability whatsoever to initiate and/or maintain the tumor [14].

Such a model of cell organization, which is the basis for the definition of a CIC, is not new. It is thought to be the rule during embryonic development, and in various adult tissues, for example, in high-turnover tissues such as the lining epithelia and the bone marrow, with a hierarchical system based on the existence of a stem cell that displays self-renewal activity through asymmetric cell division and multi-potential differentiation ability. Thus, the CIC model of tumor biology entails the existence of a hierarchy of cells within a cancer that represents a caricature of cell hierarchies existing in normal tissues. CICs are at the apex of such hierarchy [15].

The fundamental clinical implications of a tumorigenic hierarchy within a tumor become apparent when considering that anticancer therapies are not selected for their discriminatory ability to target CICs. Therefore, if a given therapy fails to eliminate all the self-renewing CICs, residual surviving CICs will be able to repopulate the disease, causing tumor relapse [16].

Although mounting evidence supports the CIC model, it is important to be aware of a number of drawbacks that should be fully addressed. First, there is no evidence whatsoever that carcinogenic cells are significantly different from non-carcinogenic cells as a result of epigenetic rather than genetic changes [17-19]. Our current understanding of tumorigenesis is that cumulative mutations develop during cancer progression [20]. Therefore, at least in theory, these changes could result in more than one CIC population capable of maintaining and propagating the tumor, by developing a self-renewal program. This theory is supported by the observation that there are neoplasias (for example pancreatic carcinoma or acute lymphoblastic leukemia, [ALL]) in which more than one phenotypically distinct population can initiate tumors *in vivo* [21]. Likewise, in acute myelogenous leukemia (AML), CICs (AML-CICs) undergo clonal evolution [22], while in chronic myelogenous leukemia (CML) one or more Imatinib-resistant CML-CIC subclones may be selected during therapy and progress to blast crisis (see below) [23-24].

Furthermore, there is evidence for chromosomal instability in the CICs derived from human metastatic colon carcinoma [25]. Based on these findings, a new CIC hypothesis should probably include both the stochastic/clonal evolution and the CIC hierarchical components [26].

Second, a lineage-committed progenitor cell can also function like a CIC by acquiring self-renewal, as demonstrated in both human CML [27] and a murine model of acute promyelocytic leukemia (APL) [28]. Third, the premise that transplanted CICs can recapitulate the heterogeneity of the tumor from which they originated is usually based on limited analyses of a few surface markers [29]. It remains to be determined if there is also genetic heterogeneity within the primary tumor, that is not present after CIC xenotransplantation. Thus, it may be that the functional and phenotypic diversity within these neoplasias are underestimated. Fourth, a fundamental issue in the CIC model is related to the very low number of the CICs. The gold standard for estimating and quantifying CICs *in vivo* is limiting dilution followed by transplantation in Non Obese Diabetic/Severe Combined Immunodeficient (NOD/SCID) mice. This method could lead to underestimating the frequency of CICs in some cancer types, such as AML and melanomas, as seeding and tumor development in animals vary widely from case to case [30-33].

It has been proposed that xenotransplantation in animals which are not entirely immunodeficient may lead to underestimating the number of CICs *in vivo*. This may also depend on the fact that the microenvironment or the CIC niches (which clearly support CIC functions) are species-specific and often tumor-specific [34]. Hence, the mouse microenvironment/niche may differ in many aspects from the cancer microenvironment/niche sustaining CICs in the natural (human) host. Moreover, murine microenvironment/ niche receptors and cytokines may not necessarily cross-interact in all cases with the respective targets expressed in human CICs [35].

In light of these findings, there is certainly a need to re-evaluate the evidence which supports the CIC model using assays that could allow for a better engraftment of human cancer cells. In 1989, Hill and Milas suggested that mouse tumors contained stem cells and that the number of these cells within the tumor could be an important determinant of prognosis [36].

However, the first evidence of the existence of CICs/LSCs came from the seminal studies performed in human AML patients by Bonnet and Dick (see below) [37]. They demonstrated that leukemic CICs were capable of serially transferring AML into an immunodeficient mouse host. AML CICs were hierarchically organized and originated from a HSC rather than a committed progenitor cell. Leukemic CIC resided in the $CD34^+/CD38^-/Lin^-$ compartment (*i.e.* they are phenotypically similar to normal SCID repopulating cells), although exceptions to this rule have been reported [38]. The existence of leukemia CICs has been subsequently demonstrated in other malignant hematological disorders, including CML [39] and T-cell acute lymphoblastic leukemia (T-ALL) [40].

More than a decade after the identification of leukemic CICs, there is mounting evidence that CICs also exist in solid tumors. These include breast carcinoma [41], prostate carcinoma [42], pancreatic carcinoma [43], colon carcinoma [44], lung carcinoma [45], brain tumors (glioblastoma and medulloblastoma) [46], and hepatocellular carcinoma (HCC) [47]. However, compared with leukemias, the issue of solid tumor CICs still remains in its infancy.

The so-called side-population (SP) is thought to be enriched in CICs. SP cells actively extrudes the nuclear acid-staining dye Hoechst 33342 owing to high expression on their plasma membrane of drug resistance transporters of the ABC family such as ABCB1and ABCG2, and can be easily identified by flow cytometry (See below) [48]. The enrichment of SP in leukemic CICs has been demonstrated in: multiple myeloma [49], T-ALL [50], AML [51], lung cancer [52], prostate cancer [53], breast cancer [54], glioblastoma [55], and HCC [56]. Intriguingly, it is emerging that in CICs, "stemness" genes are upregulated when compared with the main population. Accordingly, in the SP of liver cancer cell lines, the WNT pathway is highly expressed (See below) [57].

INCREASED EXPRESSION OF PROTEINS INVOLVED IN DRUG RESISTANCE IN LEUKEMIA CICS

Although CICs from various types of cancer have the potential for self-renewal, they spend the majority of their time in the G_0

phase of the cell cycle. This means that chemotherapeutic drugs, which act on cycling cell populations such as doxorubicin, are less effective on CICs than BCs [58]. Furthermore, the CICs are also often resistant to radiotherapy. The quiescent, non-cycling state of CICs may contribute to their resistance to conventional cancer treatments which target the rapidly proliferating cell. This quiescent nature of CICs may result in low rates of long-term remission and multi-drug resistance although, CICs also often display increased expression of drug transporter proteins.

Drug resistance can result from a plethora of mechanisms that include: decreased drug uptake, increased drug efflux, accelerated detoxification, defective apoptosis or altered expression of signaling pathways [59]. A single biochemical abnormality listed above may not be sufficient for drug resistance; there may be more than one component involved. Often these different biochemical properties can interact, either additively or synergistically and result in an increased drug resistant phenotype. ATP-dependent drug efflux is often observed clinically and is frequently linked to the increased expression of ATP-binding cassette (ABC) transporter proteins [60]. This type of drug resistance occurs frequently in patients treated with drugs such as doxorubicin (a.k.a. Adriamycin). Patients with this type of drug resistance frequently develop "multi-drug" resistance as they become cross-resistant to drugs such as doxorubicin, daunorubicin, paclitaxel, etoposide and others which can be transported by the same drug transporter [MDR1 (multi-drug resistance-1 a.k.a., P-glycoprotein, Pgp), and multi-drug resistance association protein (MRP1) and others].

The ABC family of active drug transporters contains approximately forty-nine separate transmembrane proteins, many of which can function to efflux chemotherapeutic drugs [60-61]. Some of these proteins involved in transport are functional in diverse cell types and play critical roles in normal physiology by regulating the levels of a variety of nutrients and biologically active substances across cellular barriers. That is a key reason why it has been difficult to target "drug" transporters as they also regulate the levels of other types of molecules that are essential for normal physiology. The ABC family is divided into seven subfamilies: ABCA through ABCG. Several of these genes including *ABCG1* and *ABCB1* are expressed in immature $CD34^+CD38^-$ hematopoietic cell fractions and are down-regulated upon differentiation into the more mature $CD34^+CD38^+$ sub-population.

de Grouw and colleagues demonstrated that twenty-two ABC transporters were differentially expressed in AML CICs compared to AML BC. All the ABC transporters were expressed at lower levels in the more differentiated $CD34^+CD38^+$ cells in comparison to the less differentiated $CD34^+CD38^-$ cells [62]. *ABCB1*, *ABCG2* and *ABCC1* are the three primary "multi-drug resistant" transporter genes that are expressed most frequently in tumor cells. Included in this sub-group are MDR1, BCRP (breast cancer resistance protein) and MRP1 [60]. These transporter proteins involved in drug efflux have been shown to confer drug resistance by mediating the active efflux of diverse anti-cancer drugs [63].

MDR expression is often higher in patients with secondary leukemias as compared with those in primary disease states [64]. Several potential drugs have been developed in attempts to block and compete with Pgp mediated drug efflux. Unfortunately, most of these MDR1 inhibitors have not proven effective [60,62,65-67]. Second generation MDR modulators have also yielded similar results [67]. MDR1 is not the only transporter which can efflux certain commonly-used chemotherapeutic drugs. Novel drugs that inhibit multiple transporters and other modes of drug-resistance might be more effective. Upon examination of the expression of various ABC transporters, a high redundancy was observed in both normal and early AML cells which may contain the AML CICs [62]. That is, the AML cells may overexpress more than one transporter which regulates the levels of a particular group of drugs. Current MDR modulators may be ineffective due to the expression of overlapping transporter proteins, which can efflux the same drug, thus making targeting of a single protein ineffective. Additionally, it is possible that irrelevant transporters were inhibited or there were pharmacokinetic interactions between the chemotherapeutic agent and the ABC transporter inhibitor. Third generation MDR modulators that are more powerful are being developed and examined in clinical settings [60] however, so far encouraging results have not been reported. Clearly the proteins involved in drug transport could be key targets for elimination of CICs, however, it is also evident that their targeting will be difficult as there are functional overlaps between the different transporters and these transporters also play other essential roles in physiology.

EXPLOITING INCREASED EXPRESSION OF DRUG TRANSPORTERS AND ALDEHYDE DEHYDROGENASE TO IDENTIFY LEUKEMIA CICS

The ability of CICs to efflux many drugs has been practically exploited in the isolation of CICs in different types of cancers. Many ABC transporters expressed in CICs efflux the fluorescent dyes Hoechst-33342 and rhodamine 123. This is in contrast to BCs, which retain the dyes. After Hoechst-33342 staining and flow cytometric analysis, a large percentage of CICs have been shown to reside in the SP cells. These SP cells were found in the bone marrow of over 80% of AML patients [60]. Identification of SP cells was one of the first techniques used to identify CICs from various different types of cancers.

CICs isolated from various cancer types also often express elevated aldehyde dehydrogenase activity (ALDH) and it is being used as a key marker for CIC identification [68]. AML patients who's CICs express elevated ALDH have a poorer prognosis than patients which express lower levels of ALDH [68]. Targeting of ALDH is complicated as it likely plays important functions in many different tissue types, not just CICs and there are at least twelve different ALDH isoforms.

A WELL STUDIED MODEL FOR CICS AND THERAPY—CHRONIC MYELOID LEUKEMIA (CML)

In the following section, we will discuss CML as it is a well-studied leukemia which has had significant advances in therapy in the past 15 years due to the discovery of the effective BCR-ABL inhibitor, Imatinib as well as additional second and third generation inhibitors. Furthermore, CML, unlike AML and many other cancers, has a unique chromosomal translocation, t (9;22), which is present in virtually 100% of CML patients [69]. As a result, part of the breakpoint cluster region (*BCR*) gene from chromosome 22 is fused with the *c-ABL* gene on chromosome 9. This abnormal "fusion" gene generates a protein of 210-kDa (p210) or sometimes 190-kDa (p190).

CML is characterized by the overproduction of mature myeloid cells. This leukemia is subdivided into three distinct phases: chronic phase, accelerated phase and blast crisis, which is phenotypically similar to AML. While AML CICs are biologically and functionally distinct compared to HSC, CML CICs are often phenotypically similar to normal HSCs. CML CICs cells are present in $CD34^+CD38^-$ cells and contain the *BCRABL* translocation present on what is historically referred to as the Philadelphia chromosome. CML patients have clonal expansion of hematopoietic cells that express the BCR-ABL proteins. Expression of BCR-ABL is essential for the sustained CML proliferation [70]. Current CML therapy is treatment with BCR-ABL kinase inhibitors. However acquired resistance to BCR-ABL kinase-inhibitor therapy can occur as well as a lack of sustained molecular remissions [63].

CML CICs are phenotypically $CD90^+$, $Thy1^+$ and Lin^-. Blast crisis CML patients have higher levels of the progenitor pool ($CD34^+Lin^-$ cells) than the other classes of CML patients. CML patients which are responsive to BCR-ABL targeted therapy, have fewer $CD34^+Lin^-$ cells in comparison to patients non-responsive to

BCR-ABL inhibitors. The β-catenin pathway is overexpressed in patients resistant to BCR-ABL inhibitors [27,71] and will be further discussed below. The differences in response in patients resistant to BCR-ABL kinase inhibitors as compared to those not resistant implies the acquisition of self-renewal capacity as well as other selective genetic changes in granulocyte–macrophage progenitors present in the CML CICs [27]. These results demonstrate the complex differences between CML CICs and BCs. While both cell types display enhanced proliferative capacity in comparison to normal myeloid cells, they display different growth properties in the presence of BCR-ABL inhibitors, which may be due to the presence of discrete additional mutations in the *BCRABL* gene present in the CML CICs (see below) or selection as a result of treatment.

Some of the CML CICs exhibit elevated BCR-ABL expression and changes in levels of the cytokines interleukin-3 and granulocyte colony stimulating factor (which may serve autocrine stimulatory roles), and the drug transporters ABCB1/MDR1, ABCG2 and the transcription factor Oct-1 upon culture with reduced levels of growth factors [71]. Additional studies by Jiang *et al.,* demonstrated that greater than seventy different *BCRABL* mutations were present in the progeny of cultured CML CICs [72]. This group has hypothesized that CML patients possess CICs, which have pre-existing *BCRABL* kinase mutations before the advent of BCR-ABL inhibitor therapy; hence the patients already have some resistant CML CICs. As the CML CICs proliferate slowly, the patient may be initially responsive to therapy, then given time, CML CICs that are resistant to BCR-ABL inhibitors emerge (see discussion of mathematical models for CML presented below). This karyotype evolution of resistant cells from pre-existing CML CICs, which already have mutations in *BCRABL*, is a lingering problem in BCR-ABL-directed therapy (15). Since CML CICs, like other CICs proliferate slowly, or are in a quiescent-like state, some investigators have proposed that a means to target these cells is to stimulate their proliferation and then treat with BCR-ABL inhibitors [73-74]. However, this therapeutic approach will only be appropriate if the CML CICs do not contain the T315I *BCRABL* mutation or a similar mutation, which confers resistance to many BCR-ABL inhibitors.

MATHEMATICAL MODELING PROVIDES EVIDENCE FOR CML CICS

Upon careful mathematical analysis of the CML incidence data and reappearance of the disease after discontinuation of therapy, it has been predicted that there are pre-existing CML CICs which are already resistant to BCR-ABL inhibitors such as Imatinib [75-78]. Basically upon analysis of the decrease in BCR-ABL mRNA transcripts upon and during the course of Imatinib treatment, a decrease to a low, constant level of BCR-ABL mRNA transcripts was observed as long as the drug Imatinib was effective, however once the patient was taken off Imatinib therapy, there was a dramatic, and rapid rebound in the level of BCR-ABL mRNA transcripts which actually may exceed the initial levels of BCR-ABL mRNA transcripts. These results suggest the existence of CML CICs in the CML patients which were not eliminated by the Imatinib treatment. In addition, this model provides an explanation of the biphasic decrease of the BCR-ABL transcript upon initial therapy with Imatinib. Initially Imatinib quickly eliminates the terminally differentiated leukemic cells with an average lifespan of approximately twenty days which leads to a rapid decrease in the levels of BCR-ABL mRNA transcripts. However, after elimination of these differentiated cells, the less mature leukemic progenitors with an average lifespan of 125 days under Imatinib therapy are responsible for the slower but continued decrease of the BCR-ABL transcripts. The immature CML CICs are not sensitive to Imatinib, and expand gradually over time at a slow rate and are responsible for a rapid rebound of the leukemic burden upon cessation of Imatinib treatment which can exceed the initial level. Furthermore, if there are mutations present in the *BCRABL* gene which confers resistance to Imatinib, the emergence of this clone(s) appears and levels of BCR-ABL mRNA transcripts increase even in the presence of Imatinib, again arguing for the presence of CML CICs in the CML leukemia patient. The models of Michor *et al*. also describe well the higher incidence of mutations in patients who receive therapy at a later stage of the disease. In these patients, the increased pool of CML CICs has a higher stochastic probability of mutations which then lead to a rapid expansion of resistant progenitors and treatment failure.

The quiescent nature of CML CICs may be responsible, in part, for the failure of Imatinib treatment to result in their total elimination. CML CICs may enter a dormant, Imatinib-resistant state or in some cases enter a proliferative Imatinib-sensitive state. Also CML CICs express higher levels of Pgp. Imatinib is a substrate for Pgp, thus with drug resistant CML CICs, Imatinib will not be an effective drug. Mathematical models, with various growth related parameters, have been proposed to explain the asymmetrical stem cell replication; however the biochemical mechanisms responsible for the asymmetry of CIC division are not well understood. Mutations occurring in the *BCRABL* gene are commonly thought to occur before disease onset and treatment, thus they are not believed to be responsible for the quiescence nature of the CML CICs. Cells containing mutant *BCRABL* genes may emerge later, due to the elimination of the cells with the wild type (WT) *BCRABL* gene upon Imatinib treatment. This model implies that the cells with the mutant *BCRABL* genes may not proliferate as well initially as the cells with the WT *BCRABL* gene. On the other hand, conflicting data indicate, that these mutations may confer a growth advantage, even without Imatinib treatment [79-80]. Clearly these models need further investigation. What are the key messages that we have learned from CML CICs? Many therapy-resistant CML CICs may already exist in the CML patient with key *BCRABL* mutations before therapy. After cessation of therapy these resistant CML-CICs may emerge and expand, perhaps in part due to the BCR-ABL treatment eliminating the CML BCs which were responsive to the therapy.

INTERACTIONS OF BCR-ABL WITH DOWNSTREAM SIGNALING PATHWAYS LEADING TO IMATINIB RESISTANCE--POTENTIAL NOVEL TARGETS FOR THERAPY

BCR-ABL induces multiple signaling pathways, that is probably why it is such a "potent" oncogene. Also it must be remembered that BCR-ABL is not a normal protein in the cell, it is a chimeric protein encoded by the abnormal *BCRABL* chromosomal translocation. BCR-ABL can activate the RAS/RAF/MEK/ERK, PI3K/Akt, p38MAPK, JNK, FAK, SRC and RAC signaling cascades [69, 80]. BCR-ABL can also interact with SRC-family kinase HCK which can lead to STAT5A activation that is JAK kinase-independent. This may be an important factor in the resistance of cells to Imatinib therapy (see below for section on SRC kinases) and also why it is appropriate to treat certain CMLs with SRC inhibitors such as Dasatinib. An important signaling family induced by BCR-ABL is the RAC family of GTPases which was summarized in a recent review [80]. An overview of some of the signaling pathways activated in leukemic CIC is presented in Fig. (**1**). Also presented in this figure are the sites that small molecule inhibitors and antibodies may act.

RAC is a multi-gene family which consists of *RAC1*, *RAC2* and *RAC3*. *RAC1* and *RAC3* are ubiquitously expressed while *RAC2* is expressed predominately in hematopoietic cells. BCR-ABL also interacts with RHO GTPases including RAC, RHO and CDC42. RAC-1, RAC-2 and to a lesser extent RAC-3 are detected at hyperactivated levels in HSCs and CML CICs. RAC-1 and RAC-2 cooperate with BCR-ABL and induce a myeloproliferative disease of HSC origin. Deficiency of *RAC1* and *RAC2* in specific knock-out mice reduced the severity of BCR-ABL-mediated transformation and prolonged survival was observed [81-82]. RAC-3 may play an important role in the cancer development in the *BCRABL, Rac-*

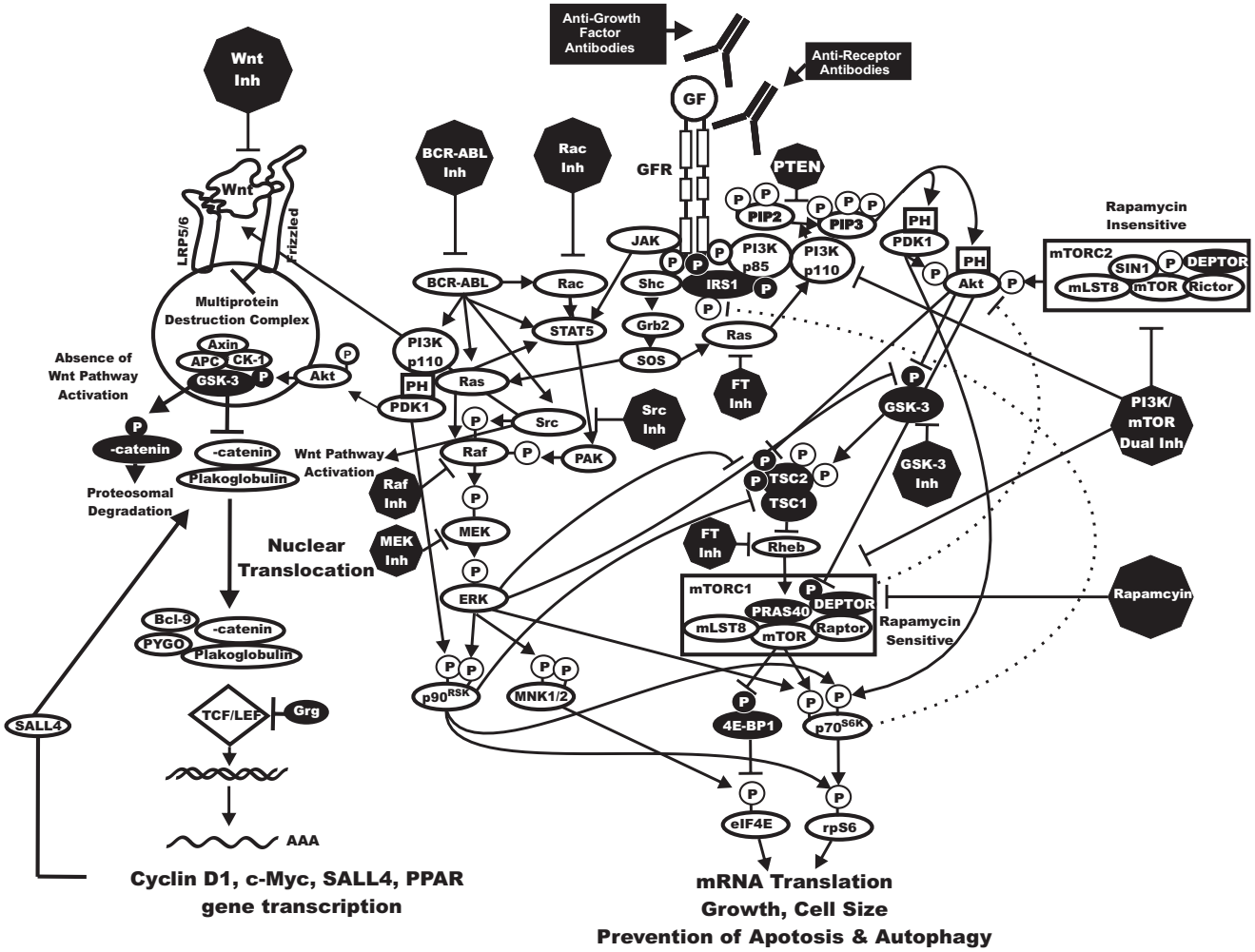

Fig. (1). Interactions between BCR-ABL, RAC, RAS/RAF/MEK/ERK, RAS/PI3K/PTEN/mTOR and Wnt/β-Catenin Pathways that Result in the Regulation of Protein Translation and Gene Transcription in Leukemia CICs and Potential Sites of Interaction by Small Molecule Inhibitors. The Ras/Raf/MEK/ERK and Ras/PI3K/PTEN/Akt/mTOR pathways can affect protein translation by complex interactions regulating the mTORC1 and mTORC2 complexes. GF stimulation results in GFR activation which can activate the Ras/Raf/MEK/ERK and Ras/PI3K/PTEN/Akt/mTOR, JAK/STAT as well as other pathways. Chromosomal translocation such as BCR-ABL can activate these pathways as well as others including RAC and SRC. AKT can phosphorylate and inhibit the effects of GSK-3β, TSC2 and PRAS-40, which result in mTORC1 activation. ERK and PDK1 can phosphorylate $p90^{Rsk1}$, which in turn can phosphorylate and inhibit TSC2. AKT-mediated phosphorylation of GSK-3β also affects the WNT/β-catenin pathway and epithelial mesenchymal transition (EMT). SRC also has effects on activation of the WNT/β-catenin pathway. Rapamycin targets mTORC1 and inhibits its activity and also results in inhibition of downstream p70S6K. The effects of rapamycin are complex as long term administration of rapamycin may prevent mTOR from associating with mTORC2 and hence full activation of AKT is prevented. However, rapamycin treatment may result in activation of PI3K, by inhibiting the effects of p70S6K on IRS-1 phosphorylation which results in PI3K and AKT activation. Also rapamycin treatment may result in the activation of ERK in some cells, presumably by inhibition of the p70S6K mediated inhibition of IRS1. These later two effects of rapamycin could have positive effects on cell growth. Inhibition of PDK-1 activity can also result in activation of mTORC1, presumably by suppression of p70S6K and hence inhibition of IRS1 effects on PI3K activity. The PTEN, TSC1 and TSC2 tumor suppressor genes all converge on the mTORC1 complex to regulate protein translation. RHEB is a small G protein important for the regulation of mTORC1 & 2 and is negatively regulated by TSC2 and is also subject to farnesylation. Thus the RAS/RAF/MEK/ERK and RAS/PI3K/PTEN/Akt/mTOR pathways can finely tune protein translation and cell growth by regulating mTORC1. Rapamycin can have diverse effects on these processes. Also these pathways can interact with the WNT/β-catenin pathway which is important in developmental processes, EMT and CICs. Upon activation of the WNT pathway, β-catenin forms a complex with Bcl-9, PYGO, plakoglobulin and TCF/LEF which result in the transcription of critical genes including cyclin D1, c-Myc, SALL4 and PPARδ. The sites where key inhibitors targeting molecules are important in these pathways are indicated in black octagons with white lettering. FT = farnesyl transferase inhibitor.

1/Rac-2-deficient mice. These results suggest an important role for RAC GTPase in BCR-ABL-mediated CML CIC generation as well as in normal HSC functions. The RAC GTPases and RAS may be important in the induction of STAT5 which may be abnormally activated by various mechanisms, including an autocrine mechanism in Imatinib-resistant CML [83].

RAC proteins play key roles in the retention of murine HSC and may be an appropriate target to eliminate CICs [82,84]. The effects of an experimental RAC inhibitor (NSC23766) on HSC mobilization have been examined [82]. This inhibitor increased HSC mobilization, suppressed RAC activation and downstream p21 activated protein kinase (PAK) activation. NSC23766 also inhibited the growth of BCR-ABL- transformed cells, even those containing the Imatinib-resistant T315I mutation [85]. This RAC inhibitor may mobilize CML CICs from their niche and thus inhibit the stem cell

properties of these cells. Hence, this inhibitor, unlike others such as Imatinib, may effectively target the CML CICs.

ROLE OF SRC FAMILY KINASES IN CML AND ALL CICS

Imatinib has proven highly effective in treatment of CML, however, Imatinib, by itself, will not cure CML. This is often due to the development of resistance in the CICs (as described above). SRC and other signaling pathways are involved in Imatinib resistance [86-87]. SRC family kinases are activated by BCR-ABL and inhibition of BCR-ABL by Imatinib may not result in the complete inhibition of SRC-family kinases. BCR-ABL is known to activate at least three SRC-family kinases (LYN, HCK and FGR) which are required for the development of BCR-ABL-mediated proliferation of preB-acute lymphoblastic leukemia (ALL), but not myeloid progenitor cells [88]. However, all three of these SRC-family kinases are activated by BCR-ABL in myeloid cells. BCR-ABL may directly interact with at least two SRC-family kinases (LYN and HCK) and alter their activities [89-90]. By performing genetic studies it was demonstrated that LYN, HCK and FYN are required by BCR-ABL to induce CML to progress to lymphoid blast crisis in mice injected with BCR-ABL-transduced bone marrow [86].

As stated previously in this review, some CML CICs carrying *BCRABL* mutations such as T315I are resistant to Imatinib, Dasatinib and other BCR-ABL inhibitors. However, in the studies by Hu *et al*., [86], they demonstrated that while BCR-ABL kinase inhibitors prolong the survival of CML mouse models, they do not completely eliminate CML CICs. In these studies, the structures of the *BCRABL* genes in CML CICs were determined and they did not have the *BCRABL* T315I mutation. These results strongly indicate that other mutations, can be involved in CML development and that Imatinib and Dastinib are not adequate to completely eliminate the CML CICs.

Alternatively, other genetic mutations occurring in blast crisis CML may contribute to the persistent activation of SRC. Mutations at other genes including, *INK4a*, *RB* and *TP53* among others are involved in CML progression [91-93]. Loss of the *ARF* gene enhances tumorigenicity of *BCRABL*-transformed cells and limits the response to Imatinib in BCR-ABL-induced B-ALL mice [94]. Studies by Hu *et al*., [86] have demonstrated that the progression to lymphoid blast crisis requires activation of SRC family kinases and the SRC kinase inhibitor Dasatinib was more effective than Imatinib in preventing B-ALL, however, eventually the disease reoccurred. Furthermore, while Dasatinib treatment prolonged the life of the CML mice, it did not eradicate CML CICs cells. The authors have therefore suggested that an additional component of BCR-ABL-expressing CML CICs must be suppressed for complete eradication of the disease. Furthermore, certain *BCRABL* mutations such as T315I are resistant to Imatinib, Dasatinib and other BCR-ABL inhibitors. Interesting, Aurora kinase inhibitors (VX-680) have shown promise in inhibiting Imatinib-resistant *BCRABL* T315I-transformed cells [95-96].

SRC kinases may induce the WNT/β-catenin and RAF/MEK/ERK signaling pathways which result in LEF/TCF activation [97-98]. Activation of these and other signaling pathways may contribute to the survival of CICs (see below).

INVOLVEMENT OF THE WNT/β-CATENIN SIGNALING PATHWAY IN LEUKEMIA CICS

The WNT signaling pathway plays a critical role in self-renewal of HSCs and its dysregulation may be involved in CIC generation. There are approximately 20 *WNT* genes in the human genome and they encode lipid-modified secreted glycoproteins [99]. WNT induces cell signaling to a receptor complex consisting of a Frizzled family receptor and a co-receptor of the LDL-receptor related protein family, usually LRP5 or LRP6. This can result in the destabilization of the multiprotein destruction complex (MDC) and the subsequent stabilization of β-catenin. β-catenin normally has a very short half life. In the absence of WNT, β-catenin is normally phosphorylated by components of the MDC including: axin, adenomatous polyposis coli (APC), glycogen synthase kinase 3β (GSK-3β) and casein kinase 1 (CK1). When β-catenin is phosphorylated by these kinases, it is targeted for proteasomal degradation Fig. (**1**).

In contrast, if cytoplasmic β-catenin is stabilized by WNT signaling (that is not phosphorylated by the MDC complex); it migrates to the nucleus and replaces the groucho-related repressors present on certain genes. β-catenin in the presence of the accessory proteins BCL-9, PYGO (pygopus) and the TCF/LEF (T-cell factor/lymphocyte-enhancer-binding factor) forms a complex which results in transcription of important growth promoting genes such as *CMYC* and CYCLIN-D1 (*CCND1*). Furthermore, TCF/LEF can result in an increase in *SALL4* expression which is an oncogene that can bind to β-catenin and enhance its activity. Plakoglobin is another important component of WNT signaling. It is a co-inducer of TCF/LEF transcriptional activation and stimulates the transcription of CYCLIN-D1, c-MYC and perxosome proliferator-activated receptor-delta (PPARδ). Plakoglobulin expression is enhanced by certain AML fusion proteins. The WNT pathway is frequently dysregulated in AML [27].

The WNT pathway is regulated by many different inhibitors which have been shown to have important roles in oncogenesis. These interactions are well described in the above mentioned comprehensive review by Mikesch *et al*., [99]. Some key inhibitors include members of the Dickkopf (DKK) family which interact with LRP5/6 and prevent the transmission of signals from the Frizzled-LRP complex. The WNT inhibitor factor 1 (WIF-1) is another inhibitor of the WNT pathway. It is an extracellular protein which binds to WNT, thereby inhibiting the WNT pathways [100]. The promoter region of *WIF1* is hypermethylated in many APLs and associated with a poorer disease free three year survival rate than in patients without WIF-1 promoter methylation [101].

Importantly, this pathway has been shown to have critical roles in self renewal of HSC and CICs [102-104]. WNT expression inhibits the differentiation of HCS [104-105]. Overexpression of axin, a component of the MDC, inhibits WNT signaling and in turn this results in decreased growth of HSC [102]. Constitutive overexpression of activated β-catenin increased the frequency of HSC and prevented their differentiation [102].

Specific deletion of β-catenin (*CTNNB1*) in hematopoietic lineages in mice did not prevent the formation of HSC, however, the HSC were deficient in long-term growth and maintenance [103]. The target(s) for CML CICs and BCR-ABL-transformation were greatly reduced while the induction of ALL occurred fairly normally. These and other results suggest that ALL may arise from a more mature stem cell committed to the B cell lineage. Conditional deletion of β-catenin (*CTNNB1*) decreased the self-renewal ability of CML CICs. Thus β-catenin was necessary for the increased self-renewal capacity conferred by the introduced *BCRABL* oncogene on the target stem cells to permit transformation to proceed along the myeloid lineage. The authors demonstrated that BCR-ABL phosphorylation was decreased in the β-catenin-deficient mice which also resulted in lower levels of phosphorylated STAT5a. BCR-ABL and β-catenin form a complex and the authors have suggested that this complex may stabilize BCR-ABL [106]. Reduction of the BCR-ABL:β-catenin complex could result in a decrease in the phosphorylation of BCR-ABL targets such as STAT5a and decrease CML self renewal and disease progression. These important results provide further evidence of the role of β-catenin and the WNT pathway in normal HSC as well as CML CICs and demonstrate the stem cell origin of CML but not ALL. WNT signaling may be critical for BCR-ABL stability and the maintenance of CML CICs. The WNT pathway may serve to differentiate CML and ALL. The successful development of WNT inhibitors may serve to augment the ability of BCR-ABL inhibitors to eradicate CML.

WNT inhibitors are being developed by several pharmaceutical and biotechnological companies; however, there do not appear to be any peer-reviewed papers documenting their effectiveness.

Not unexpectedly, the WNT signaling pathway interacts with other signaling pathways including Notch, sonic hedgehog and PI3K/AKT [106-108]. Increased FLT-3 signaling in AML patients with mutations/amplifications of *FLT3* may result from AKT mediated phosphorylation and inactivation of GSK-3β which results in higher levels of WNT signaling and stabilized β-catenin [109]. Alternatively, Frizzled-4, a WNT receptor, is induced by certain *FLT3* mutations which lead to increased β-catenin levels that result in augmented TCF/LEF activity and c-*MYC* transcription [110-111].

INTERACTIONS BETWEEN CICS AND THEIR MICRO-ENVIRONMENT AND POTENTIAL MOLECULES FOR TARGETING

Whether CICs also depend on their particular niche for self-renewal is currently unclear. While there are clear differences between normal stem cells and CICs, there are also striking similarities. Some molecular mechanisms that enable self-renewal in normal stem cells may exist in CICs.

Indeed, both normal HSC and leukemic CICs depend on stromal cell derived factor-1 (SDF-1)-mediated CXCR4 (CXC chemokine receptor-4 which is specific for SDF-1) signaling for homing and mobilization [112]. WNT-induced β-catenin signaling has been implicated in the maintenance and expansion of murine HSCs, whereas inhibition of the β-catenin pathway severely impaired the self-renewal capacity of CML CICs [27,102,113]. The adhesion of normal $CD34^+$ stem/progenitor cells to bone marrow stroma and fibronectin is mediated by the integrins VLA-4 and VLA-5, and a similar role is fulfilled by integrins in leukemic cells [114-115]. Thus, many of the molecules that mediate the interaction between stem cells and the bone marrow niche are utilized by both normal HSC cells and CML CICs.

Whether the leukemic CIC needs collaborative genetic hits to improve their interactions with the extrinsic stem cell niche remains an intriguing question. One possibility is that CICs migrate more when compared with normal HSCs that allowing the CICs to escape growth inhibition or quiescence promoting signals induced by osteoblasts and stromal cells in the niche. Many membrane-associated ligands are normally present in the niche, and they can be cleaved by matrix metallo-proteinases (MMPs). MMPs and their tissue inhibitors (TIMPs) are important in the progression and invasiveness of many malignant disorders. The expression of MMPs such as MMP9 is often elevated in AML blasts [116]. MMPs may increase the soluble concentrations of activated ligands to enable leukemic CIC self-renewal or expansion outside the niche. Therefore, the level of marrow MMP-9 may be a useful surrogate marker for monitoring disease status in AML and it was proposed as a potential prognostic factor. Targeted inhibition of MMPs may inhibit CIC expansion and may prove useful in leukemia therapy.

Targeting CD44 with a monoclonal antibody could suppress both AML and CML progression and induce differentiation [117-119]. The monoclonal anti-CD44 Ab may disrupt interactions between the CICs and the bone marrow niche. RAC inhibitors may also disrupt these interactions and mobilize LSC (see below).

SUPPRESSING CICS: TARGETING THE PI3K/PTEN/AKT/MTOR PATHWAY

The PI3K/PTEN/Akt/mTOR pathway, and specifically PTEN levels, may be critical for the development of CICs [120-137].

The concept that the PI3K/PTEN/Akt/mTOR pathway serves as a therapeutic target in leukemia CICs is beginning to emerge. CICs have unique properties as they can be both quiescent and also resistant to chemotherapeutic and hormonal based drugs. However, under certain conditions, they resume proliferation and hence could be potentially susceptible to PI3K, AKT or mTOR inhibitors.

The *PTEN* gene exerts effects on CICs, especially in hematopoietic and breast cells. In conditional *PTEN* knock-out mice, upon inactivation of *PTEN*, there was a transient increase in hematopoietic CICs and a myeloproliferative disease develops and the mice subsequently developed leukemia after 4-6 weeks [120]. If the mice were treated with the mTOR inhibitor rapamycin, the myeloproliferative disorder and leukemia were prevented. The initial leukemic CICs that arose after conditional *PTEN* deletion by themselves were not able to induce leukemia upon transfer into SCID-recipient mice, but if the leukemic CICs were derived from the *PTEN*-conditional mice that had developed leukemia, they were able to transfer leukemia to the SCID-recipient mice, which could be prevented by rapamycin treatment. Also the normal HSC from the *PTEN*-conditional knock-out mice repopulated the hematopoietic cell component of irradiated mice treated with rapamycin indicating that it was possible to selectively eliminate leukemic CICs. However, rapamycin treatment did not eradicate the PTEN-deficient leukemia CICs after leukemia onset.

PTEN plays critical roles in regulating cell cycle progression in HSC and other cells. PTEN influences the decision of whether the cells remain in quiescence (G_0) or enter G_1 and also controls the subsequent speed of proliferation [128]. Importantly PTEN influences hematopoietic differentiation and PTEN-deficiency blocks differentiation of B lymphoid stage resulting in an increase in the presence of myeloid and T cells but a decrease in cells of the B-lineage.

Additional studies have identified some of the genes which can interact with *PTEN* deletion to influence leukemogenesis [130]. The authors demonstrated that *PTEN*-deletion induced the expression of $p16^{Ink4a}$ and TP53 in the HSC and $p19^{Arf}$ and TP53 in other hematopoietic cells. Functional TP53 suppressed leukemogenesis in *PTEN*-deficient mice and also promoted HSC depeletion. While $p16^{Ink4a}$ also stimulated HSC depletion, it had a limited role in suppressing leukemia development. In contrast, $p19^{Arf}$ strongly suppressed leukemia development but did not decrease HSC levels. The authors demonstrated that in the *PTEN*-mutant mice, secondary mutations inhibited the tumor suppressor response. Therefore in the leukemic clones that arose in these *PTEN*-deficient mice, mTOR activation depleted HSC by a tumor suppressor response which was inhibited by secondary mutations. These authors did not observe a role for reactive oxygen species (ROS) in the depletion of HSCs in *PTEN*-deficient mice. In contrast, other studies have suggested roles for ROS in depletion of *TSC1*-deficient HSCs [131]. In these studies, TSC1 depletion led to mTOR activation, exit from quiescence, increased proliferation but resulted in exhaustion of HSCs without the development of leukemia.

Other genes in this pathway also have been shown to have effects on HSC depletion. *TSC1*-deficient mice result in increased PI3K/PTEN/Akt/mTOR signaling which leads initially to increased HSC cell cycling and mobilization then to progressive depletion and defective long term repopulation [132]. These authors also demonstrated that TSC1 regulation of HSC mobilization is also effected by mTORC1-independent mechanisms and that one of the genes involved is the actin-bundling protein FSCN1 (fascin). Fascin is believed to be an important mTORC1-independent mediator of the effects of TSC1 on the regulation of HSC mobilization. FSCN1 plays important roles in controlling cytoskeleton organization and motility and is upregulated in many human cancers. Fascin promotes cell migration and invasion [133]. TSC1 regulation of fascin may play important roles in the dissemination of leukemia CICs. This is an interesting observation as in solid tumor CICs; a frequent observation is the formation of sphere-like cells (*e.g.*, mammospheres) and masses. FOXO is also an important regulator of HSC homeostasis. FOXO is essential for long-term HSC regeneration.

FOXO regulates quiescence and survival in response to oxidative stress [134-135].

SUPPRESSING CICS: TARGETING PML

Targeting of PML also leads to increased HSC cycling and to mTOR-mediated HSC depletion [136]. The *PML* gene is involved in the t(15:17) chromosomal translocation found in acute promyelocytic leukemia (APL). *PML* encodes a protein which localizes to PML nuclear bodies. PML is a tumor suppressor and controls many processes including apoptosis, proliferation and senescence. PML is reported to have diverse biological functions and is a negative regulator of the AKT/mTOR pathway at multiple levels including opposing the nuclear function of AKT, repressing mTOR activity by inhibition of RHEB/mTOR in hypoxia and regulating PTEN localization through modulation of a deubiquitination network [128]. PML is highly expressed in HSCs and there are more PML nuclear bodies in the HSC than in the committed cell populations. PML expression decreases as the HSC differentiate. PML also has consequences on the outcome of CML. CML blasts express high amounts of PML which is regulated at the transcriptional level. PML loss or lower expression predicts a better prognosis in CML [128,137]. Recently it has been shown that PML is a negative regulator of mTOR [137]. PML is important in HSC maintenance and $PML^{-/-}$ HSC display increased cycling compared to HSC from normal mice, but the $PML^{-/-}$ HSC lack long-term repopulating capacity which is likely due to defective maintenance of quiescence. Conditional ablation of *PTEN* in the hematopoietic system of mice drives exit from quiescence, exhaustion of normal HSC, and generation of leukemia CICs *via* activation of the PI3K/Akt/mTOR pathway. Likewise loss of PML enhances the cycling pool of HSCs though mTOR activity. However, an important difference between $PML^{-/-}$ and conditional ablation of *PTEN* is that $PML^{-/-}$ mice do not develop leukemia, whereas in mice where *PTEN* is conditionally-ablated, leukemia develops [128]. The exit from quiescence is more profound in leukemia CICs from $PML^{-/-}$ mice than in HSC from $PML^{-/-}$ mice, indicating a potential difference which could be exploited for therapy. Rapamycin opposes leukemogenesis in *PTEN*-conditional knock-out mice and restores normal HSC function. In contrast, rapamycin restores leukemia CIC maintenance in the $PML^{-/-}$ model.

PML expression is high in leukemia CICs [136]. PML is very important for leukemia CIC maintenance. Leukemia CICs from $PML^{-/-}$ mice were generated by infection with a retrovirus encoding BCR-ABL. Similar to HSC isolated from $PML^{-/-}$ mice, leukemic CICs from $PML^{-/-}$ mice undergo intensive cell cycling, but displayed reduction of long-term transfer of CML disease (in the third serial bone marrow transplant), failed to generate minimal residual disease and impairment of leukemia CIC maintenance.

Arsenic trioxide (As_2O_3) selectively targets PML for degradation. As_2O_3 specifically targets KSL (c-Kit^+, $Sca\text{-}1^+$, Lin^-) cells and reduced their colony forming ability but did not affect KSL cells isolated from $PML^{-/-}$ mice.

Increased mTOR activity was observed in HSC prepared from $PML^{-/-}$ vs. WT mice. Rapamycin treatment restored the colony forming ability of $PML^{-/-}$ HSC and rescued the phenotype of $PML^{-/-}$ HSCs and leukemia CICs [136]. Rapamycin increased the quiescence of $PML^{-/-}$ HSCs. PML has an important role in maintenance of HSCs by repressing mTOR activity. Rapamycin also had effects of $PML^{-/-}$ leukemia CICs. It restored their ability in long term colony formation assays. PML downregulation eliminates leukemia CICs.

As_2O_3 reduced PML levels in leukemic CICs and decreased the number of quiescent CIC in the absence of the induction of apoptosis [128,136-137]. Consistent with these results, the long-term maintenance of the CIC was dramatically suppressed. The authors examined the effects of cytosine arabinoside (Ara-C) and As_2O_3 on leukemia CICs. They hypothesized that interventions that increase the cycling of quiescence CIC might enhance their death by chemotherapeutic agents. The combined treatment resulted in eradication of the CICs even after 4 weeks after termination of treatment and long term repopulating assays could not detect the presence of CICs or the presence of minimal residual disease.

Importantly the authors demonstrated that the HSC and leukemic CICs display some important differences. Namely there were fewer quiescent cells in BCR-ABL- expressing CICs than in control KSL HSC, suggesting that the reservoir of quiescent cells is higher in normal HSC than in CICs [128,136-137]. Furthermore the As_2O_3-induced exit from quiescence was more profound in the CICs than in the HSC. The authors also confirmed some of these important observations on CICs and HSCs isolated from human CML patients and normal volunteers respectively. These studies indicate the potential therapeutic approaches of combining PML-reducing drugs and chemotherapeutic drugs for certain leukemia therapies.

The PI3K/PTEN/Akt/mTOR pathway may mediate HSC activity through AKT/FOXO instead of mTOR [134-135]. PML may oppose mTOR as well as AKT/FOXO and hence is a critical regulator of HSC and leukemia CICs.

EFFECTS OF AGE OF CICS ON MALIGNANT POTENTIAL

Recently is has been shown in certain animal models that there is a correlation between the age of the induction of leukemia and the rapidity of development of the leukemia [138]. In these studies it was determined that the age of the recipient mice did not alter the potential for leukemia after injection of *BCRABL*-transformed BaF/3 cells, namely, both young and old mice developed leukemia at approximately the same time. However, in a conditional *BCRABL*-mouse model (CombitTA-BCR-ABLp190) where the expression BCR-ABL could be regulated by controlling the presence or absence of doxycline (tetracycline-repressor system), it was observed that leukemia (B-ALL) was more rapid when BCR-ABL was induced by removing doxycline from the drinking water at either 12 or 20 months of age than when BCR-ABL was induced in 4 months of age. This may result from the selection of CICs with a more potential malignant phenotype in the older mice. Alternatively there may be more complimenting mutations in the CICs present in older mice which can interact with BCR-ABL to result in more aggressive CICs than in the CICs present in younger mice.

CANCER INITIATING CELLS IN SOLID CANCERS

CICs have been identified in many different solid tumors including, breast, prostate, colon, hepatocellular carcinoma, pancreatic, glioblastoma, melanoma and others [122-129, 139-143]. Some of these CICs (*e.g.*, colorectal CICs) express one or multiple cell surface markers associated with stemness such as CD122, CD44, CD24, CD29, CD166 and Lgr5 [139]. The targeting of certain of these molecules may eliminate CICs [139]. The PI3K/PTEN/Akt/mTOR Pathway has been shown to be important in multiple cancer types including breast cancer [121-122, 143-144]. Targeting this pathway may be an appropriate approach to eliminate CICs.

CONCLUSIONS

CICs are thought to be the essential fraction of the tumor that is responsible for the integrity of the tumor. That is, the CICs can re-establish the tumor after removal of the original tumor by chemo- or radio-therapy or surgical approaches. Experimentally, the CICs can transfer the tumor more effectively to recipients than the bulk of the more differentiated tumor mass. CICs differ from BC as the CICs have retained certain essential properties of stem cells and the CICs are normally dormant or slowly replicating while the BCs are more rapidly proliferating. Importantly the CICs can undergo differentiation and give rise to the differentiated cells present in the original cancer as well as therapy-resistant cells. Therapy-resistant

cells may result from mutations that occur in the CICs during the original cancer treatment, or they may be a sub-population of CICs which already contain the mutation and hence are selected for during the therapy. The key dilemma with CICs is that they are often refractory to most common anti-cancer approaches such as chemo-, radio-, or hormone based therapies. However, CICs may show some sensitivity to certain novel therapies. Development of novel approaches to target CICs could significantly enhance cancer therapy. In summary, the specific therapeutic targeting of leukemia CICs is a field in its infancy. The tumor cell microenvironment or niche may also be an important therapeutic target and Rac inhibitors as well as various anti-integrin antibodies may be appropriate. Recently the WNT-β-catenin and PI3K/PTEN/Akt/mTOR and PML pathways have been shown to be important in leukemia CICs. Targeting these pathways may enhance leukemia therapy. As we learn more about the various leukemic CICs, it undoubtedly will result in novel ways to treat leukemias. Certain natural products commonly used in traditional medicine may prove effective in interacting with chemotherapeutic drugs to target leukemia CICs. Drugs such as As_2O_3 may target critical proteins involved in CIC proliferation. Leukemia CICs pose challenges to classical chemotherapeutic approaches due their slow rate of proliferation and quiescent-like properties. Additionally, the increased expression of ABC transporter proteins and various cell-signaling pathways further adds complexity to the situation. However, their pivotal role in the overall control and propagation of a variety of cancers and some initial responses to various inhibitors and treatments shows their therapeutic potential. Ultimately, previous research has shown that we must be clever and multi-faceted in our approach to design therapies, which may be effective in selectively eliminating these leukemia CICs.

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DISCLOSURE

This manuscript is an updated version of our previous review article [126] published in 2009 and contains significant new information as well as new scientific references.

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